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General Commentary

Tolerability of risk: A commentary on the nitrosamine contamination issue

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ABSTRACT

For decades, regulators have grappled with different approaches to address the issue of control of impurities. Safety-based limits, such as permissible daily exposure (PDE), acceptable intake (AI), threshold of toxicological concern (TTC) and less than lifetime limits (LTL) have all been used. For many years these safety-based limits have been recognized as virtually safe doses (VSDs). Recently, however, many regulatory agencies are seeking to impose limits for N-nitrosamine impurities, which are significantly below the VSD. This commentary will discuss the evolution of safety-based limits for impurities, provide an overview of the valsartan N-nitrosamine contamination issue and review the toxicology of N-nitrosamines. The outcome of a lessons-learned exercise on sartan medications undertaken by the European Medicines Agency (EMA) will also be discussed. The review will also highlight the many analytical challenges inherent with controlling impurities to ppb-based limits. The use of highly sensitive, low ppb limits, methods may lead to future issues of batch rejection, based on false positives. Regulators initially viewed the N-nitrosamine risk as being insufficient to prompt immediate product discontinuation and patients were specifically advised to continue using their affected medication. Patients were also informed that exposure to N-nitrosamines is extremely common via food and drinking water.

Introduction

Impurities and degradation products that are by-products of the manufacture and storage of medicinal products afford no benefits to the patient, only potential risk[1] with certain exceptions including closely related impurities in proteins, peptides, or semi-synthetic products, e.g. oligonucleotides, beta-lactams, etc., where these analogues frequently retain beneficial biological activity and are often controlled as families or classes of impurities.[2] Regulators have long grappled with different approaches to address this intrinsic issue. Strategies have included risk-based assessments, e.g. ICH Q3D(R1),[3] ICHM7(R1),[4] avoidance, e.g. N-nitrosamine impurities,[5] as low as reasonably practicable (ALARP),[6] or safety-based limits, including permissible daily exposure (PDE) [employed for residual solvents in ICH Q3C(R6)[7] and elemental impurities in ICH Q3D(R1)[3]], threshold of toxicological concern (TTC), less than lifetime (LTL) limits or acceptable intakes (AIs) [used for mutagenic impurities in ICH M7(R1)[4]]; and sometimes a combination of all of these approaches [ICH M7 (R1)[4]].

However, many industry commentators have reflected that since some level of impurities is an inevitable outcome of the synthesis of complex, multifunctional active pharmaceutical ingredients (APIs), the complete avoidance of risk from impurities is not feasible.[6] Similarly, whilst meaningful measures are implemented to minimize degradation within a drug product during storage, breakdown products are still likely to be encountered owing to the unavoidable presence of moisture and oxygen within the storage environment, leading to the two most common degradation pathways, i.e. hydrolysis and oxidation.[8] As such, there needs to be an underlying acceptance that there will be low, but controlled levels of residual impurities/degradation products arising from the synthesis of drug-substance or drug-product manufacture and storage, i.e. a 'tolerability of risk' approach.[9] Toxic impurities, particularly those with mutagenic potential, pose special challenges, but even here a combination of risk-based strategies and virtually safe doses (VSDs), i.e. TTC, LTL or AI, can be used to safely control the risk to patients. On the other hand, managing extremely toxic impurities, such as some N-nitrosamines, does challenge the VSD orthodoxy and may reintroduce concepts such as avoidance and ALARP that were employed prior to the inception of ICH M7(R1),[4] i.e. the period between 2009 and 2014.[6] This review will focus on the evolution of safety-based guidance for

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impurities [with a special focus on those belonging to the Cohort of Concern (CoC), including an evaluation of their metabolism and toxicology]. In addition, discussion is focused on what happened during the N-nitrosamine contamination crisis, the reasons for its occurrence and measures undertaken to address the issue. A critique of EMA's lessons-learned review and a commentary on some of the proposed changes to existing guidance will be provided. Finally, the review will discuss whether the responses to the crisis are proportionate given the significant daily exposure to N-nitrosamines from diet and drinking water and whether the apparent reluctance to advise patients to discontinue impacted medicines is appropriate.

Evolution of safety based guidance for pharmaceutical impurities

Impurities

Impurities are controlled mainly by quality guidelines within the framework of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH): i.e., ICH Q3A(R2),[10] Q3B(R2),[11] Q3C(R6),[7] Q3D(R1),[3] Q3E,[12] Q5C,[13] Q6A,[14] Q6B.[14] Other guidance is contained in multi-disciplinary guidelines, i.e., ICH M3(R2)[15] and M7(R1),[4] and impurities in anti-cancer medications are controlled by reference to ICH S9.[16,17] ICH Q3C(R6)[7] was the first of the impurity guidelines that provided safety-based guidance on allowable limits of common residual solvents. This guideline uses either PDE limits and/or the corresponding concentration-based limits, i.e. ppm - but the ppm limits are more restrictive being based on a standard dose of 10 g . Interestingly, Class 1 solvents ("to be avoided") use only ppm-based limits, i.e. benzene < 2 ppm; but some commentators have argued that a PDE-based limit would introduce more flexibility and consistency.[18] Class 2 solvents ("to be limited") use specific PDE and ppm limits interchangeably, i.e. acetonitrile 4.1 mg/day (PDE) or 410 ppm. Finally, Class 3 solvents ("low toxic potential") use a default class-based PDE capped at 50 mg/day, corresponding to a concentration limit of 5000 ppm limit. Actual PDEs for some Class 3 solvents are many multiples of the class-based PDE; for example; acetic acid 3200 mg/day; acetone 210 mg/day; formic acid 180 mg/day; heptane 840 mg/day. Nevertheless, the concept of ALARP is sometimes introduced by quality reviewers for Class 3 solvents (although not specifically mandated by regulatory agencies); process-capability arguments may be used to reduce class 3 solvent levels well below the ICH Q3C(R6)[7] safety- and/or class-based limits.[17]

ICH Q3D(R1)[3] defines limits of residual elemental impurities. ICH Q3D(R1)[3] also uses PDEs and establishes values for different routes of administration, i.e. oral, inhalation and parenteral, and introduces a risk-based approach. There is no expectation to tighten PDE limits based on process capability considerations provided that PDE values are not exceeded. However, specific elements can be removed from the drug substance specification if they can be shown to be absent routinely at levels equivalent to 30% of the designated PDE [cf. ICH Q3C(R6)[7] and ICHM7(R1)[4]].

Finally, ICHM7(R1)[4] defines limits of mutagenic impurities (MIs) within a drug substance or drug product. MIs are controlled on the assumption that they can potentially cause cancer in man. A TTC approach, originally based on food safety considerations, was developed to describe an acceptable intake for any novel MI. This equates to a generic allowable exposure of 1.5 μg/day based on a carcinogenic risk of 1 in 100,000, which is viewed as a VSD. The control strategies for MIs are based on established risk-assessment approaches.

The acceptable excess risk of carcinogenic potential is considered greater for early development phases, i.e. 1 in 1,000,000, because the acceptable risk in healthy human volunteers is lower than that in patients, although the total exposure duration is limited, i.e. LTL. Thus, for example, if the acceptable intake is 1.5 μg/day for lifetime exposure, the LTL limits can be increased to a daily intake of 120 μg (< 1 month), 20 μg (> 1–12 months) or 10 μg (> 1–10 years' treatment duration). These LTL limits are applicable to both clinical development and for commercial products. In the latter case, ICH M7(R1) provides guidance in note 7 on those drug classes that can in theory utilize LTL limits. For example, the 120 μg/day limit for treatment duration of < 1 month; can be used for those drugs used in "emergency procedures (antidotes, anaesthesia, acute ischaemic stroke), actinic keratosis, and the treatment of lice". In contrast, for commercial products, the lower cancer risk level of 1 in 100,000 is considered acceptable for lifetime exposure, i.e. 70 years. MIs are then classified into five different categories in order of decreasing regulatory concern:

- Class 1, known mutagenic carcinogens. Control at or below compound-specific limit, i.e. AIs or PDEs. For example, hydrazine has an AI of 39 μg/day, based on a TD_{50} linear extrapolation (ICHM7 (R1)) and aniline has a threshold-based PDE of 720 μg/day. Interestingly, AIs but not PDEs can be adjusted using the LTL factors.
- Class 2, known [or (Q)SAR-predicted] mutagens with unknown carcinogenic potential. Control at or below allowable limits, i.e. LTL or TTC.
- Class 3, contain alerting structures (not related to API) with no supporting mutagenicity data. Control at or below allowable limits, i.e. LTL or TTC. Or conduct bacterial mutagenicity assay; if non-mutagenic = Class 5, if mutagenic = Class 2.
- Class 4, contain alerting structures related to known structures or the specific API, which are non-mutagenic. Treat as non-mutagenic impurity, i.e. use default ICH Q3A(R2)[10]/Q3B(R2)[11] limits.
- Class 5, contain no alerting structures, or alerting/mutagenic but non-carcinogenic. Treat as non-mutagenic impurity, i.e. use default ICH Q3A(R2)/Q3B(R2) limits ICH M7(R1)[4] includes four control options for API MIs; of these only one stipulates control of the MI in the API specification (option 1). Options 2 and 3 identify some levels of in-process control; in contrast, option 4 is focused on process understanding alone, typically purge-factor arguments.[19]

The control strategies for MIs in oncology products are different since the latter are outside the scope of ICHM7(R1)[4] given that the risk/benefit ratio in advanced-cancer patients differs from that in the normal population. As such, MIs in oncology products can be controlled at higher levels (normally based on ICH Q3A(R2)[10]/3B(R2)[11] criteria). For other indications when the drug substance is itself genotoxic at therapeutic concentrations and may be expected to be associated with an increased cancer risk, a similar approach on impurities is recommended in ICH M7(R1).[4] Conversely, this pragmatic risk/benefit based approach is not routinely applied to other (rare) diseases where life expectancy is low.

Finally, a sub-class of MIs described as the CoC, including N-nitroso compounds, aflatoxin-like and alkyl azoxy compounds, were deliberately excluded from TTC or AI-based control strategies as they were considered to be highly toxic (typically potent mutagenic carcinogens). This is despite the fact that not all N-nitrosamines, for example N-nitrosodiphenylamine and N-nitroso-2,6-dimethylpiperidine, are mutagenic. In addition, Thresher et al.[20] recently concluded that nearly one-fifth of N-nitrosamines were considered to be non-carcinogenic in nature.

Metabolic activation of N-nitrosamines

N-Nitrosamines are generally considered to be indirect-acting mutagens requiring cytochrome P450 (CYP2E1) activation by hydroxylation of the carbon atom in the α-position relative to the nitroso moiety.[21] There are some reports indicating that radical mechanisms are involved in the initial α-hydroxylation reaction.

Further reactions produce an alkyl diazonium ion that is thought to be the proximate mutagen.

Toxicity of N-nitrosamines

Dose-response functions in genetic toxicology are often reported to be non-linear and thresholded, which applies for example to direct-acting alkylating agents, polyaromatic hydrocarbons (PAHs), aneugens and topoisomerase inhibitors.[22-27] Indeed, ICH M7(R1)[4] recognized this fact: "The existence of mechanisms leading to a dose response that is nonlinear or has a practical threshold is increasingly recognized, not only for compounds that interact with non-DNA targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage." The point of departure (PoD) approach is a useful technique for defining acceptable exposure limits in humans.[28] The PoD is the point on a dose-response curve established from experimental data from which extrapolation may be employed, in conjunction with the application of uncertainty factors, for low-dose risk assessment and determination of an acceptable exposure level, or reference dose. The $BMDL_{10}$ (benchmark dose modelling) approach, leading to a determination of an appropriate PoD, is considered a more realistic starting point for excess cancer risk assessments than the methodology using linear extrapolation of the dose that results in a 50% increased tumour incidence over background (TD_{50}).[4] The latter approach assumes a straight line from the TD_{50} to the background cancer frequency at zero dose, and is considered to be more conservative and therefore tends to overestimate the real risk; but the TD_{50} approach (based on values listed in the CPDB - Carcinogenic Potency Database)[29] – has become the preferred potency metric for risk assessments under ICH M7(R1).[4]

Initially, most agencies set AI-based interim limits for N-nitrosamines (NDMA and NDEA) calculated in accord with ICHM7(R1)[4] using TD_{50} metrics. A read-across approach was utilized for some N-nitrosamines considered to possess similar alkyl substituents, i.e. NDIPA, NEIPA, NMBA. For other N-nitrosamines, i.e. MNP, CPNP, a lower class-specific limit of 18 ng/day (derived from the Lhasa carcinogenic potency database) can be used as a default option, although this limit is extremely conservative given that the N-nitrosamine with the highest potency, NDEA, has a higher AI limit of 26.5 ng/day)[30] (see Table 1 for naming, abbreviations, structures and allowable intakes of the various N-nitrosamines).

The CHMP in their updated questions-and-answers update of the article 5(3) review have also indicated, without a clear explanation, that the use of LTL limits, a mainstay of ICH M7(R1),[4] particularly in early drug development, is not permitted for N-nitrosamines.[30]

Linear extrapolation

The risk assessments for NDMA and NDEA to date have been based on the default linear-extrapolation approach of the cancer potency data.[31] Due to the variation in response in each dose group, it is common for both the linear and non-linear models to provide a comparable fit to the data. With these substances, we are fortunate to have a 'mega-study' (rodent carcinogenicity bioassay) for each,[32-33] in that an extended dose range was tested, with an increased number of replicates per dose (15 doses plus control at $n = 60$). However, even with such an extensive data set, the conclusion of the expert authors in Zeilmaker et al.[34] is that the latent variable models (LVM-E4) that has linear behaviour at low doses, and log-probit model which is sub-linear at low doses, both adequately describe the available dose-response data. This illustrates that even for these excellent datasets, it cannot be decided whether the dose-response at the lower end is in fact linear or not.[34] Note also plotting on a log-scale produces sublinear curves (where the slope increases with dose) at the low range, whereas the curves are linear on the untransformed dose scale.[34] [The plot shown in Fig. 1 using a log-transformed dose scale seems to suggest that liver tumour incidence at the lower end of the dose scale is essentially equivalent to the spontaneous incidence in untreated animals.]

Due to these intricacies of dose-response modelling, it was considered prudent to assess these data further. The Zeilmaker et al.[34] risk assessment was focused on utilizing the data for a margin of exposure (MOE) calculation. This is an improved assessment compared to the linear back-extrapolation approach.

The conclusion of linear low-dose modelling for this data set is interesting. The BMD approach is used to apply a set of suitable models to the data, in order to define a small but measurable increase above the background or a specified increase in incidence.

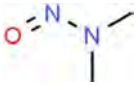
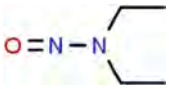
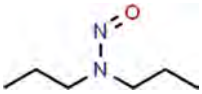
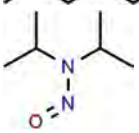
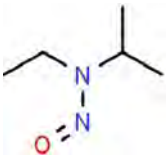
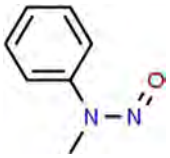
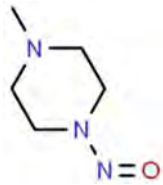
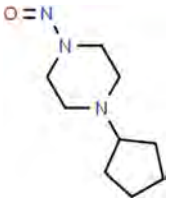
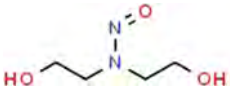
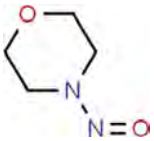
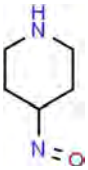
Interestingly, when the CHMP's SWP (Committee for Medicinal Products for human use Safety Working Party) reviewed the issue of safe levels for the N-nitrosamines in November 2018 they were initially divided.[35] Many committee members (the risk minimization sub-group) thought that control of N-nitrosamines could be approached in a similar manner to any other mutagenic impurity, based on the extremely conservative approaches already utilized in ICHM7(R1).[4] There was no particular evidence that NDMA and NDEA were "fundamentally different from other mutagenic carcinogens, which are covered by the TTC framework in ICH M7(R1),[4] besides being more potent". This increased toxicity can be evaluated by "defining compound-specific thresholds based on carcinogenicity data and by linear extrapolation" resulting in an AI – the strategy subsequently adopted for the interim safety-based limits (see Table 1). As such, there is no requirement for a "no threshold" approach. However, the more conservative part of SWP (the risk avoidance sub-group) deemed that "there is no need to establish AIs for impurities in the first place as the contamination risk is considered to be avoidable by avoiding certain manufacturing processes".[35] However, as ongoing reform of the "Delaney Clause" in the US highlighted,[36] total avoidance or absence of any contaminant is totally impracticable[6]; and as regulatory agencies have come to realize this, they have needed to put in place limits to control N-nitrosamines in medicinal products. However, rather than continue to use the interim AIs, which are recognized as virtually safe doses, they have implemented methodology-based limits that are entirely driven by analytical capability and unrelated to classical safety-based limits – a clear case of not tolerating even minimal risk (see N-nitrosamines methodology).

In addition to the numerous short-chain alkyl N-nitrosamines that have been identified during this crisis, two N-nitrosovalsartan impurities were also identified. Both were found to be Ames-negative, presumably because metabolic activation is significantly impaired by steric hindrance. Other Ames-negative N-nitrosamines include L-proline and hydroxyproline derivatives.

Clinical safety

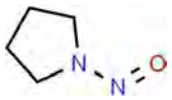
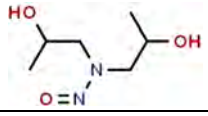
The contamination of sartans and other medicinal products with N-nitrosamines was possibly the principal medicine-related public-health episode of the last decade and the biggest issue since the heparin contamination scandal of 2007.[5,37, 38] What concerned and puzzled industry in equal measures was how did this issue occur and why the risk was not identified earlier? N-Nitrosamines are part of the so-called CoC (in fact a subset of N-nitroso compounds). According to perceived wisdom, the structural classes within the CoC were unlikely to be seen during the routine synthesis of active pharmaceutical ingredients (APIs).[39] In addition, broad, risk-based assessment processes, to evaluate the potential for formation of mutagenic impurities, were in place throughout industry [ICH M7(R1)[4]]. Therefore,

Table 1
Allowable Intakes (AI) for N-nitrosamines.[30]

Chemical Name	Structure	Abbreviation	Allowable Intake (ng/day)
N-Nitrosodimethylamine		NDMA	96.0
N—Nitrosodiethylamine		NDEA	26.5
N-Nitroso-N-methylamino butyric acid		NMBA	96.0
N—Nitrosodipropylamine		DPNA	26.5
N-Nitrosodiisopropylamine		DIPNA	26.5
N—Nitrosoethylisopropylamine		EIPNA	26.5
N-nitroso-N-methylaniline		NMPA	34.3
1-methyl-4-nitrosopiperazine		MNP	96.0¨
1-cyclopentyl-4- nitrosopiperazine		CPNP	90.0*
N-Nitrosodiethanolamine		NDELA	NA*
N—Nitrosomorpholine		NMOR	NA*
N-Nitrosopiperidine		NPIP	NA*

(*continued*)

Table 1 (*Continued*)

Chemical Name	Structure	Abbreviation	Allowable Intake (ng/day)
N—Nitrosopyrrolidine		NPYR	NA*
N-Nitrosodiisopropanolamine		NDiPLA	NA

NA: AI is not currently available.

* a class specific TTC for N-nitrosamines may be applicable.[30]
This is used in those cases where no compound-specific limit can be developed or when a (Q)SAR approach cannot be used 30 ¨ FDA quoted a ppm limit for MNP of 0.16 ppm in rifampin (rifampicin in EU), taking the maximum daily dose of 600 mg this equates to an AI of 96 ng/day, i.e. 600 mg x 0.16 ppm; ⁂ FDA quoted a ppm limit for CPNP of 0.10 ppm in rifapentine, taking the maximum daily dose of 900 mg this equates to an AI of 90 ng/day, i.e. 900 mg x 0.10 ppm.

the announcement in June 2018 that the EMA was "reviewing medicines containing valsartan drug substance supplied by Zhejianjg Huahai following the detection of an impurity" came as a significant surprise to many,[40] particularly, as the impurity of concern was found to be an N-nitrosamine, i.e. NDMA (N-nitrosodimethylamine) and this impurity had formed as "a result of a change in the manufacturing process" during the period between 2011 and 2013.[41–43]

The principal initial message to patients from world-wide regulatory agencies was that medicines containing valsartan (which is used for high blood pressure and heart failure) from specific companies were being recalled; that patients should be given alternative treatments, but it was essential that they not discontinue their medicines unless directed to do so by healthcare practitioners. Importantly, patients were told that there was no immediate risk and that there was a higher risk from terminating treatments. A frequent complaint from patients and patient groups was the absence of clear and unambiguous information from regulatory agencies' websites or indeed

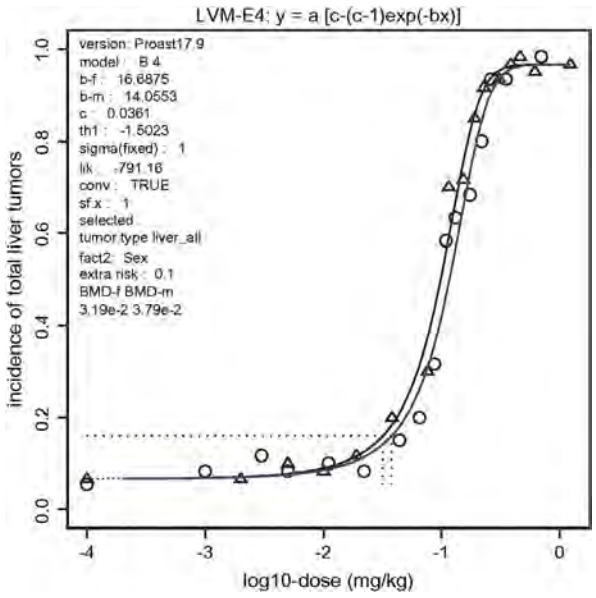


Fig. 1. Dose-response relationship for total liver tumors after lifelong exposure to NDMA via drinking water (milligram per kilogram per day). Data: Peto et al..[32] Lines: fitted LVM-E4 model for males and females, assuming parameters a and c identical but b different amongst sexes. The horizontal dotted line indicates the 10% extra risk level (compared with the estimated background response of 7.3%), the two vertical dotted lines the associated doses (BMDs) for females (triangles) and males (circles). Extra risk is defined as additional risk divided by the non-affected fraction in the control. Note that dose is plotted on log-scale, resulting in sublinear curves at the low range, which are however linear on the untransformed dose scale (Zeilmaker et al.).[34]

from healthcare professionals "as to whether their own medicines were affected".[31] This concern was shared by community pharmacists who were critical of the absence of detailed instructions for healthcare professionals from regulatory agencies, and particularly the lack of alternative treatments in the event of recalls being initiated. FDA[44] also sought to downplay the risks of N-nitrosamine contamination as they indicated in early August 2018, that "NDMA is a known environmental contaminant. For context, it is found in water and foods including meats, dairy products and vegetables". Consumer data from Finland and The Netherlands[45] show that typical adult dietary exposure to NDMA from food and beverages is around 100 ng/day (cf. AI limit of 96 ng/day), thus providing a MOE of 16,200 (> 10,000) based on the SCCS's (Scientific Committee on Consumer Safety) reference point.[5]

In early August 2018, EMA's CHMP provided an initial risk estimate for patients exposed to NDMA via contaminated valsartan from Zhejiang Huahai.[40] They indicated that: 'Following a preliminary evaluation, EMA estimates that there could be one extra case of cancer for every 5000 patients taking the affected medicines at the highest valsartan dose (320 mg) every day for 7 years. This is based on average levels of this impurity detected in the active substance from Zhejiang Huahai (60 parts per million).' FDA also issued similar reassurance stating there "could be one extra case of cancer for every 5000 patients over 4.6 years".[46] FDA updated this risk to one extra case of cancer for every 8000 patients over 4.6 years in early 2019.[47] In August of the same year, FDA further downgraded the risk, stating that "In reality, the vast majority of patients exposed to NDMA through ARBs (angiotensin II receptor blockers) received much smaller amounts of the impurity than this worst-case scenario, and, since not all ARBs are affected, it's very likely that a patient taking an ARB for four years would not have always received one of the affected products".[48] Global regulatory agencies also put the risk in the context of the lifetime risk of cancer in their territories and highlighted NDMA potential exposure from food and water sources.

During 2019, EMA published an updated risk assessment based on a potential "worst case scenario", where they assessed joint exposure to the highest observable levels of NDEA for 4.6 years, between the years 2011 to 2015, and to NDMA for 6 years, between the years 2012 to 2018. This resulted in a cumulative theoretical excess risk of cancer of 29.5/100,000 or 1/3390, i.e. 0.029% using ICH M7(R1) approaches.[49] This was then compared to the lifetime risk of cancer in the EU of approximately 50%, and on that basis, this excess risk was considered to be very low. Excess risk refers to the excess rate of cancer associated with exposure to these substances.

Most recently, FDA have strongly re-affirmed the underlying risk/benefit message, linked to contamination of two antibacterial drugs used to treat tuberculosis. FDA indicated that: "To mitigate or avoid shortages and to help ensure patients have access to these necessary

medicines, FDA will not object to certain manufacturers temporarily distributing rifampin containing 1-methyl-4-nitrosopiperazine (MNP) or rifapentine containing 1-cyclopentyl-4-nitrosopiperazine (CPNP) above the acceptable intake limits until they can reduce or eliminate the impurities". However, FDA made it clear that levels of MNP must be below 5 ppm in rifampin and rifapentin must contain no more than 14 ppm of CPNP. This is compared to the AI levels for these N-nitrosamines that are 0.16 ppm of MNP and 0.1 ppm of CPNP, respectively. Tellingly, FDA reiterated that, "FDA will not object to these higher exposures to maintain patient access to these life-saving medications" and "... the risk of not taking the medicine outweighs any potential risk from MNP or CPNP".[50]

However, there was still confusion and alarm amongst patient groups. One Canadian study[51] showed increased non-adherence following valsartan recalls with commensurate higher levels of hospitalization. The authors stated that, 'Patients may have been willing to risk the short-term potential of uncontrolled hypertension to avoid ingesting a potential carcinogen.'[51]

A recent Danish epidemiological study[52] reported on the outcome of a clinical study assessing the increased cancer risk from patients taking valsartan products that were potentially contaminated with NDMA. The final study cohort included over 5000 people, who were followed for an average of 6 years. The authors presumed any valsartan process changes leading to NDMA contamination were introduced during the period of 2012/2013. They further assumed that all subsequent medicinal products supplied post-process change would be likely contaminated with NDMA, i.e. a worst case scenario. The study found 104 cancer outcomes in the placebo group and 198 in the active group. The adjusted hazard ratio for overall cancer findings was 1.09 with no indication of a dose-response relationship between the various active groups ($P = 0.70$). For individual cancer outcomes, increased cancer risk was seen for colorectal and uterine cancers. The authors recommended that longer-term studies were needed to fully assess any long-term increased cancer risk. A British Medical Journal (BMJ) editorial[53] also called for longer-term assessment of affected patients. However, CHMP did not support these proposals.[31] Firstly, they contended that "theoretical risk of cancer was very low and was itself based on a worst-case scenario". Secondly, cancer screening methodologies carry additional risks for patients. Thirdly, there was still "considerable uncertainty as to which organs or tissues could be at risk from cancer".[3]

Valsartan contamination issue

Evolution of a crisis

NDMA contamination of valsartan products led to a rapid EU product recall of affected batches from the Chinese generic supplier, Zhejianjg Huahai (ZHP).[54] EMA findings of NDMA contamination of valsartan API from ZHP had world-wide ramifications. The Taiwanese Food and Drug Administration (TFDA) notified other regulators of the detection of NDMA in valsartan APIs manufactured by two other Chinese generic companies, Zhejiang Tianyu (ZTP) and Zhuhai Rundu Pharma (ZRP) Pharma. Based on information received from EMA, FDA reported issues with Valsartan from the same Chinese supplier, ZHP.[55] On 9 August 2018, the FDA also confirmed an N-nitrosamine recall of valsartan medicinal products from Hetero Labs Ltd in India.[56] This was the first indication that the N-nitrosamine issue was truly global as these products were only sold to US consumers. By the end of August 2018, FDA indicated that sixteen suppliers of Valsartan API to the US market had been implicated.

As a result of this flurry of findings and ad hoc inter-agency communications, an international 'Angiotensin II Receptor Blockers (ARB) International Strategic Group' was formed.[57] This group comprised Health Canada (coordinator), the EMA, US FDA, Japan's Ministry of Health, Labour and Welfare/Pharmaceuticals and Medical Devices Agency (MHLW), Australia's Therapeutic Goods Administration (TGA), Singapore's Health Science Authority (HSA), and Swissmedic. The role of the group was to coordinate activities within the various regulatory agencies and to ensure that there was visibility and awareness of each other's activities. The main areas of collaboration included the determination of risks, assessment of testing methodology, GMP site inspections, and aligned public communication. Efforts were also made to lessen duplication by having regulators conduct either concurrent or joint regulatory inspections. The FDA and Health Canada performed several concurrent inspections of sartan manufacturing sites in affected areas, whilst Health Canada raised the potential of participating in an EU inspection. Although this international strategic group was highly effective in coordinating international efforts, the absence of the regulators from India [Central Drugs Standard Control Organization (CDSCO)] and China [National Medical Product Administration (NMPA)] was, in retrospect, a deficiency.[31]

Then, in late August 2018, ZHP informed European regulators of the presence of a second N-nitrosamine, NDEA, in some batches of its valsartan API. Thereafter, the European regulatory network was informed on 14 September of trace amounts of NDEA, in another sartan, losartan, from Hetero Labs in India. EDQM (European Directorate for the Quality of Medicines and Healthcare) informed European Agencies on 17th September 2018 of the detection of traces of NDEA, in irbesartan, from another Indian generic API manufacturer, Aurobindo Pharma Limited. This was quickly followed by identification of NDEA contamination in others sartans, i.e., losartan and irbesartan, from two Indian generic companies, Hetero Labs and Aurobindo Pharma Limited, respectively.[31] In parallel, FDA reported the presence of NDEA in batches of valsartan API. FDA subsequently found low levels NDEA in Irbesartan from ScieGen[58] and losartan in a combination product, losartan/hydrochlorothiazide manufactured by Sandoz.[59]

This action prompted European regulatory agencies to risk assess all tetrazole-containing sartans, i.e. valsartan, candesartan, irbesartan, losartan and olmesartan.[60] In mid-September 2019, EMA sent a formal request to all MAHs for medicinal products "containing chemically synthesized active substances requiring them to evaluate the risk of N-nitrosamines being present in their products and to take appropriate risk mitigation measures".[61] Health agencies across the globe followed suit, with requests to evaluate the risk (step 1), of the presence of N-nitrosamine impurities in all human pharmaceutical products currently on the market; i.e. EMA, 26 Sep 2019 (Article 31: Sartan containing tetrazoles),[62] Health Canada 02 October 2019,[63] Swissmedic 15 November 2019.[64] MAHs (Marketing Application Holders) were given 6 months to redress this problem, but because of Covid-19 considerations this was extended to 12 months. Step 2 was confirmatory testing of those products at high risk of contamination and step 3 was implementation of any modifications to the marketing authorisation to minimise the issue. The interim limits for the assessment were the AIs derived from ICHM7(R1).[4] WHO also issued an update on the N-nitrosamine issue recommending similar risk-based strategies to those outlined above.[65] A transition period of two years was established within EU, i.e. up to 02 April 2021; thereafter a technical-based limit of 30 ppb will be implemented.

EMA also highlighted issues of NDMA contamination of pioglitazone manufactured by Hetero Labs in India during January 2019 – the first non-sartan to be implicated in the crisis.[61] No recalls were implemented as the levels were below the interim AI. This incident was also the first time an N-nitrosamine was found in an API where its formation did not occur in the final synthetic step; nitrite sources would need to be carried over to the subsequent synthetic stage, before reacting with a secondary amine – probably arising from dimethylformamide (DMF), a solvent used in the process. Other possible root causes were also evaluated, including solvent recycling,

which potentially could lead to DMF being contaminated with NDMA. EDQM reviewed all CEP (Certificate of Suitability to the monographs of the European Pharmacopoeia) applications and in April 2019, both EMA and NCAs (National Competent Authorities) within EU requested that all pioglitazone Marketing Authorisation Holders (MAHs) that were using specified reagents, e.g. $NaNO_2$ in their manufacturing processes check their products and processes for N-nitrosamine contamination.[66]

In March 2019, FDA identified a new N-nitrosamine impurity, N—Nitroso-N-methyl-4-aminobutyric acid (NMBA) in losartan from Hetero Labs in India.[67] During April 2019, FDA sought to re-assure US patients by indicating that there were 40 ARB (angiotensin II receptor blocker) medications where FDA assessments had concluded that they do not contain any known nitrosamine impurities.[68]

In the middle of 2019, a fourth N-nitrosamine, N-nitrosomethylphenylamine (NMPA) was found in valsartan from another Indian generic manufacturer, Divi's Labs. The levels observed were below the proposed AI for NMPA.[31] In September 2019, Valisure issued a Citizens Petition in the US relating to ranitidine. They indicated that "Valisure tests all batches of all its medications for quality and consistency issues and through such tests detected extremely high levels of N-nitrosodimethylamine ("NDMA"), a probable human carcinogen, in every lot tested, across multiple manufacturers and dosage forms of the drug ranitidine".[69] They contrasted the typical levels of NDMA found in ranitidine tablets of 3000,000 ng/ tablet, i.e. 3 mg/tablet, with the AI limit of 96 ng/day of NDMA for this potent mutagen. FDA conducted a review and did indeed find elevated levels of NDMA, but these levels were much lower than reported by Valisure and this was attributed to a methodology issue (see N-Nitrosamine Methods).[70] During September 2019, EMA also initiated an Article 31 review for all ranitidine products after testing demonstrated NDMA contamination.[57] This resulted in several EU national authorities initiating product recalls. On 12 September 2019 following the detection of NDMA in excess of the interim AI, all CEPs for ranitidine hydrochloride were suspended by EDQM.[71] There is some limited evidence that NDMA may form from the degradation of ranitidine itself with higher levels seen over its shelf life. It is still not clear whether NDMA can also be formed via metabolism in vivo. EMA has therefore decided that, "Given the uncertainties, the CHMP has recommended a precautionary suspension of these medicines in the EU".[71]

Based on all of these events, EMA arrived at the view that an Article 5(3) review for other medicines was required. In mid-September 2019, EMA a sent a formal request to all MAHs for medicinal products "containing chemically synthesised active substances requiring them to evaluate the risk of N-nitrosamines being present in their products and to take appropriate risk mitigation measures".[61] As a result of these findings in several different classes of medicinal products, Health Agencies across the globe initiated requests to evaluate the risk (step 1), of the presence of N-nitrosamine impurities in all human pharmaceutical products currently on the market.

EMA initiated a separate Article 5(3) review (step 2, i.e. confirmatory testing) assessment of metformin products in late 2019. FDA indicated that they were aware of low levels of NDMA in metformin drug products, highlighted by other regulatory agencies, but that the levels were "within the range that is naturally occurring in some foods and in water.[67] FDA subsequently analysed metformin tablets from 16 batches from seven different companies and reported that all batches were in compliance with FDA's limit for NDMA.[73]

Valisure issued a second Citizens Petition relating to high levels of NDMA that they had found in metformin products.[74] FDA subsequently conducted a review, and found lower levels of NDMA than reported by Valisure in metformin API and various drug products. The issue was again methodology-related (see N-nitrosamine methods).[75] However, FDA did find levels of NDMA above the AI in metformin extended-release products and they subsequently requested voluntary recalls of these products.[76] The issue was attributed to the drug product from five suppliers and not the input API. Other manufacturers of metformin extended-release products were not impacted.

In the early part of 2020, FDA found NMDA in a second H2-receptor blocker drug, i.e. Neftazidine.[77] In June 2020, Health Canada acknowledged the increased likelihood for NDMA formation in doxylamine succinate API.[78] Historically, NDMA had been found in doxylamine succinate antihistamine preparations.[79]

EMA completed the review under Article 5(3) (i.e. EC 726/2004) in June 2020 that provided guidance to MAHs on how to avoid the presence of nitrosamine impurities in human medicines. CHMP asked MAHs in July 2020 to assess all chemical medicinal products, and for the first time, all biological medicinal products used in man for the potential presence of N-nitrosamines and to test affected products at risk following the CHMP guidance. This review of biological products complements the similar review of chemically synthesized active substances (ongoing since September 2019).[80] In the middle of 2020, EMA published a very comprehensive "Lessons Learnt" review. The main findings are critiqued separately in this commentary.

In the latter half of 2020, a new class of drugs (rifamycin antibiotics, i.e. rifampin (rifampicin in EU) and rifapentine) were implicated and a further two, novel, N-nitrosamines, i.e. MNP and CPNP were found. In parallel, the EU also investigated the presence of MNP, in some batches of drug substances used in rifampicin drug products.[81]

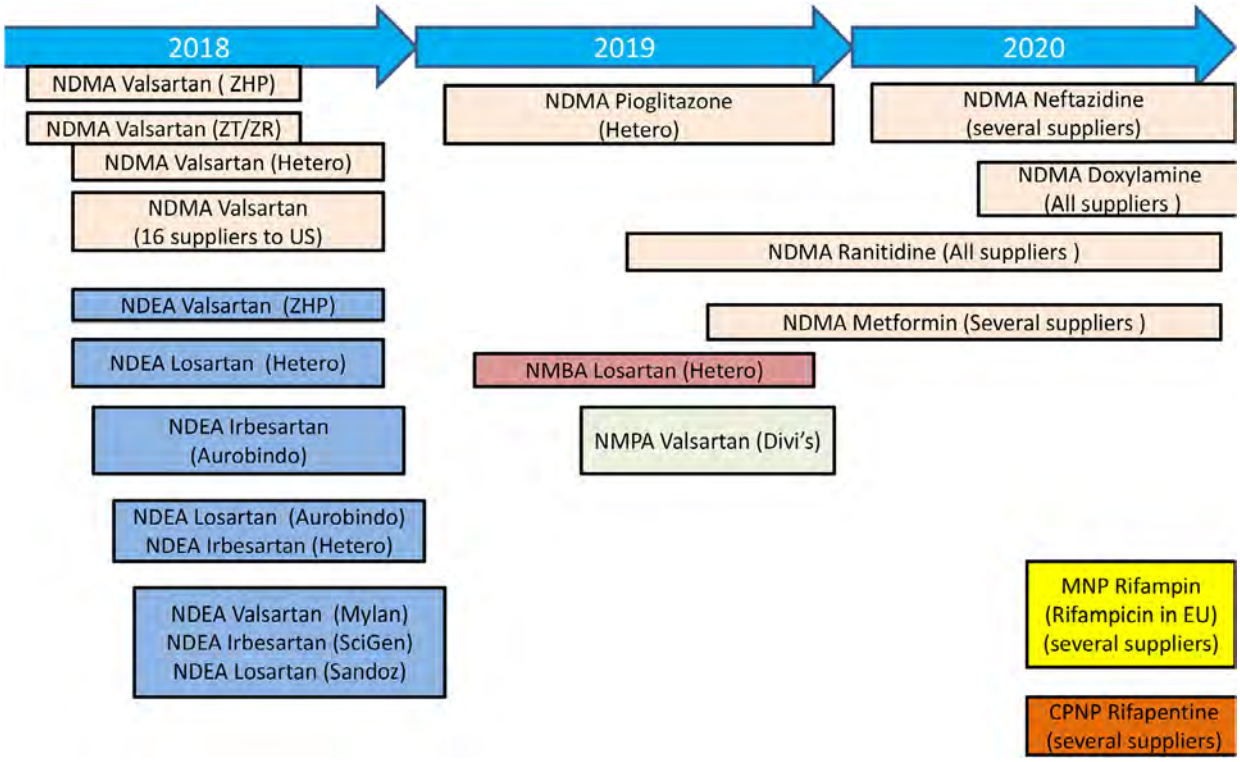
A schematic of the different types of N-nitrosamines identified thus far, in different medicinal products, i.e. sartans, pioglitazone, metformin and anti-tuberculosis (TB) products, as well as the different suppliers (where known) is provided in Scheme 1.

For both of these tuberculosis drugs, FDA clearly articulated that, "…the risk of not taking the medicine outweighs any potential risk from MNP or CPNP". FDA, allowed higher exposures of these N-nitrosamines (i.e. higher than their designated AIs) "to maintain patient access to these life-saving medications". FDA also indicated that "they would determine on a case-by-case basis whether these drugs should be released for distribution".[83] EMA gave similar guidance, "The risk to patients from not taking their rifampicin medicines far outweighs any potential risk from MNP. Healthcare professionals should therefore continue to prescribe rifampicin medicines as normal in accordance with the product information".[84]

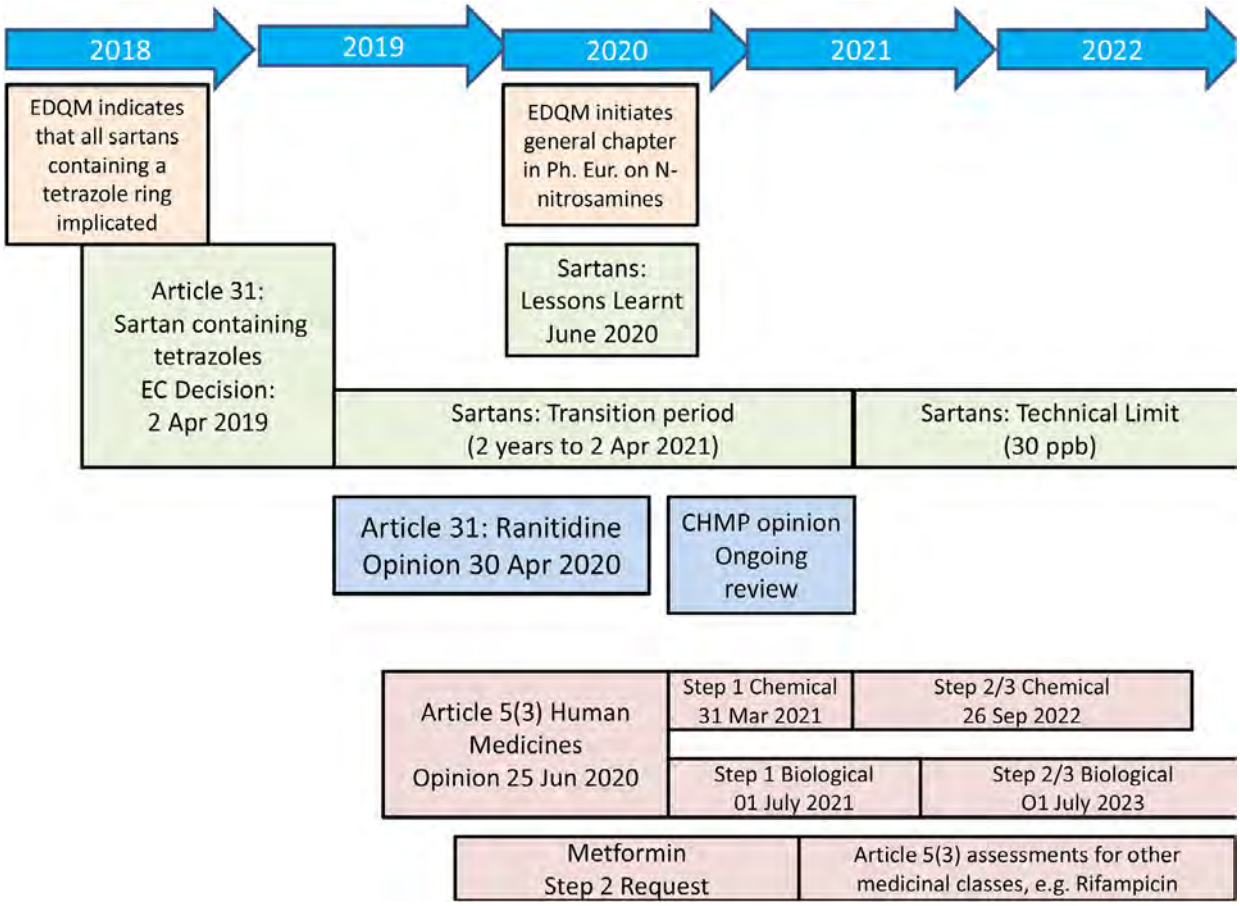
EMA also indicated that they will address any future issues arising from findings in other drug classes, e.g. metformin[85] and rifamycin[84] antibiotics under separate article 5(3) reviews. A schematic of the EU regulatory response to N-nitrosamine crisis is given in Scheme 2; as it can appear confusing to non-EU nationals.

How did this contamination occur? As there were no N-nitrosamine issues with the originator valsartan product, Diovan, attention rapidly focused on the synthetic processes used by ZHP. This generic company replaced tributyltin azide with sodium azide in the tetrazole ring-formation reaction. This, in turn, necessitated the use of $NaNO_2$ as an azide-quenching agent (see Scheme 3).[86]

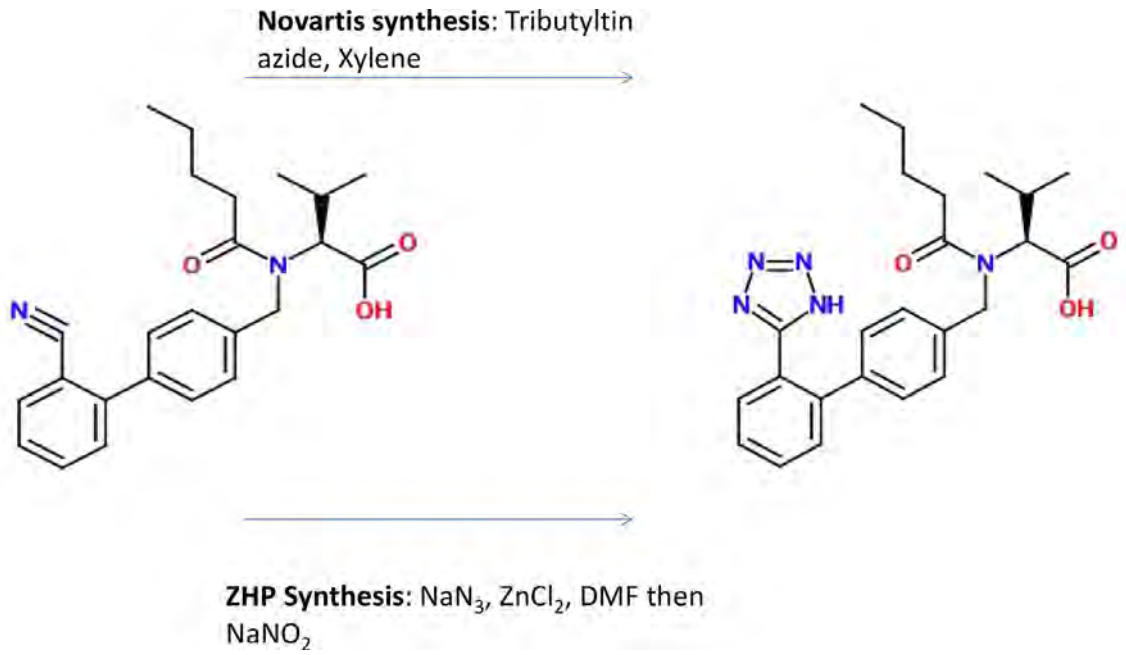
However, excess nitrite under acidic conditions can also form nitrous acid. This was then free to react with secondary amines, e.g. dimethylamine, diethylamine, to form the corresponding N-nitrosamine, i.e. NDMA or NDEA, respectively. It would appear that these volatile dialkyl amines are formed as impurities in DMF (dimethylformamide) which is utilised as the solvent in the reaction. Although, the thermal instability of DMF is well documented in the literature,[87] it was not assessed in the mutagenic risk assessment. Since the tetrazole ring-forming reaction is the penultimate stage of valsartan synthesis, there was little opportunity to purge these volatile N-nitrosamines. In contrast, if the tetrazole ring system is introduced earlier in the synthesis, as with candesartan, there is greater opportunity to effectively remove these impurities,[88] even when they need to



Scheme 1. Timelines for identification of various N-Nitrosamine impurities (impurity type, product type and supplier).[82]



Scheme 2. EU regulatory response to N-nitrosamine crisis.



Scheme 3. Comparison of penultimate stage of synthetic pathway for valsartan (ZHP vs. Novartis).[86]

be controlled to very low levels, i.e. NDMA, AI of 96 ng/day and NDEA, AI of 26 ng/day.

In contrast, levels of NDMA in ranitidine were likely due to the inherent thermal/hydrolytic instability of ranitidine; as the molecule contains both nitrite and dimethylamine (DMA) fragments, then NDMA could form due to intra-molecular rearrangements. Nizatidine is similarly unstable and can form NDMA on storage.[89] N-Nitrosamines other than NDMA and NDEA, arising from different alkyl amines have also being reported, i.e. NMBA.[90] Recently, FDA reported that certain rifamycin antiobiotics, i.e. rifampin and rifapentine have shown N-nitrosamine contamination leading to two additional analytes, i.e. MNP and CPNP.[83]

It is now abundantly clear that the N-nitrosation reaction can occur between nitrous acid (or residual nitrites in an acidic environment) and any small chain alkyl secondary or tertiary amine; indeed, in some cases reactions have been observed with amine drug substances, e.g. N-nitroso valsartan.

This is summarized in Table 2.

In addition, outsourcing of recycling of solvents, reagents or catalysts by third parties resulted in additional N-nitrosamine contamination, i.e. all good manufacturing practice (GMP) deviations. FDA issued a warning letter to Lantech Pharmaceuticals Limited in Telangana, India, for GMP violations. Lantech is a contract solvent recovery facility used for many valsartan drug substance manufacturing operations. FDA determined that recovered solvent was contaminated with NDEA; the company had not assessed the risks linked with their processes and did not effectively assess those impurities.[72] However, as a result of contamination arising during recycling there has been a significant increase in waste solvents, with commensurate environmental implications.

NDBA and NMPA were also found to arise from their corresponding amines, which in turn could be impurities in the phase transfer catalyst, tetrabutylammonium bromide (TBAB). Finally, contamination also originated from unsatisfactory cleaning and cross-contamination in large multi-purpose facilities. FDA recently sent a warning letter to Maylan, Andhra Pradesh, India for "failure to adequately clean equipment and utensils", linked with the ARB-contamination issue.[91] Another cause of N-nitrosamine contamination has been linked with primary blister packaging. The root-cause assessment identified reactions between amine components within the printing ink and nitrocellulose in the lidding foil leading to the formation of N-nitrosamines. These volatile N-nitrosamine impurities could then contaminate drug product during the heat-sealing part of blistering operations. As nitrocellulose lidding is a very common component in blistering operations it could potentially impact on many products. However, there is a relatively easy fix for this problem by replacing nitrocellulose lidding components and the levels of NDMA/NDEA found were below the alerting limits.[31]

N-Nitrosamine contamination has been linked to residual NDMA levels in water; that is, potable, pharmacopoeial-grade and other bespoke grades, i.e. water for injection. NDMA contamination can arise as a by-product of disinfection processes using chloramination, i.e. the reaction of monochloramine with dimethylamine, a common contaminant in water. It appears that ranitidine (and other members

Table 2
Source of Amine leading to N-nitrosamine formation.[31]

N-nitrosamine	Source of Amine
NDMA	Degradation of DMF to DMA followed by N-nitrosation
NDEA	De-alkylation of DIPEA followed by N-nitrosation
	Disproportionation of TEA.HCl to yield TEA followed by de-alkylation and subsequent N-nitrosation
	DEA impurity in TEA, and subsequent N-nitrosation
DIPNA	Dealkylation of DIPEA followed by N-nitrosation
EIPNA	Dealkylation of DIPEA followed by N-nitrosation
NMBA	Ring opening of NMP to yield MBA followed by N-nitrosation
NMPA	Dealkylation of N,N-DMA followed by N-nitrosation
NDBA	Nitrosation of DBA
	Dealkylation of TBA followed by nitrosation
MNP	TBD
CPNP	TBD

N-NITROSAMINES: NDMA: N-nitrosodimethylamine; NDEA: N-nitrosodiethylamine; NMBA: N-nitroso-N-methylamino butryic acid; NMPA: N-nitrosomethylphenylamine; NDBA: N-nitrosodidibutylamine; DIPNA: N-nitrosodiisopropylamine; EIPNA: N-nitrosoethylisopropylamine. **SOLVENTS**: DMF: dimethylformamide; DMA: dimethylamine; DIPEA: Diisopropylethylamine; TEA: triethylamine; DEA: diethylamine; MBA: 4-methylaminobutryic acid; N,N-DMA: N,N- dimethylaniline; DBA: dibutylamine; TBA: tributylamine. TBD To be decided, investigations are still underway.

of its class) show the most significant potential for NDMA formation from water that was disinfected with chloramine and ozonolysis.[92] In addition, NDMA can be formed as a by-product of chlorination.

NDMA can also arise as a by-product of anion-exchange treatment for water softening. NDMA is generally destroyed by ultraviolet irradiation during water treatment. The existing WHO guideline for drinking water ascribes a limit for NDMA of 0.1 ppb arising from different sources in the environment.[93-94] Some parts of Europe and the US have in place lower limits. As a result of its high aqueous solubility, it is very unlikely that potable or process water constitutes a significant source of NDMA contamination for APIs. Similarly, water-based processes in secondary manufacturing, i.e. wet granulation, film-coating, spray drying, lyophilisation or preparation of aqueous solutions are deemed to be low risk, due to the extremely low levels of N-nitrosamines.[31]

The final cause of potential contamination arises from residual nitrites in water[95] and excipients.[96]

These residual nitrites could subsequently react with amines (APIs or impurities), reagents and many solvents, to form N-nitrosamines. Drinking water, and other types of water, are likely contaminated with nitrite. The WHO has assigned a limit for nitrite in drinking water of 3 mg/L (3 μg/g or 3 ppm) as nitrite ion. Nitrosation of amines in APIs or impurities would be very slow due to low reagent concentration and high activation energy. As such, suitable conditions for N-nitrosamine formation would require elevated temperatures and acidic conditions.

N-Nitrosamines arising from water are not considered to be a likely source of contamination of drug substances. Nitrosation arising from degradation processes in API/drug products are theoretically possible but extremely unlikely. Disinfected water currently cannot be eliminated as a causative factor, but it seems unlikely. Ashworth et al.[95] used established "mechanistic and kinetic studies of amine nitrosation to assess the risk that traces of nitrite in the water" utilized during drug substance manufacturing could generate significant amounts of N-nitrosamines. They concluded that the concentrations of nitrite in water, that is used for drug substance manufacture are very low, i.e. <0.01 μg/mL, and hence will not generate significant levels of N-nitrosamines by reacting with basic secondary amines with pKa values greater than 9.5.

Nitrites as reactive impurities are likely to occur in many excipients at ppm levels.[96] These include lactose fast flo^R, PVP (polyvinyl pyrrolidone), pre-gelatinised starch, croscarmellose sodium and sodium starch glycollate. Nitrites probably arise during processing.

When reviewing the N-nitrosamine contamination issue holistically, the only example of where suspicion is focused on the product (rather than the API) are the Metformin ER (extended release) medications. No issues were seen with the API or corresponding metformin IR (instant release) products, only the ER products. As such, residual nitrites in the polymeric excipients reacting with amine sources in the formulation could be the cause, but at this stage that is conjecture. Similarly, it is not currently clear how the rifamycin antibiotics, i.e. rifampin and rifapentine are contaminated with MNP and CPNP, respectively.

N-Nitrosamine analytical method

European union

The Official Medicines Control Laboratories (OMCL) network within the EU reacted rapidly to the sartan crisis. In retrospect it does seem strange that there was initial confusion over the intended analytical target range.[31] Snodin & Elder[5] commented that it was illogical that many of the initial OMCL methods were not aligned with one another with respect to limit of quantification (LOQ), when it was clear that the AI for NDMA in valsartan was 0.3 ppm, i.e. 96 ng/day. By September 2019, six methods had been developed by the OMCL consortium.[31] None of the OMCL laboratories initially published method validation as per ICH Q2(R2). As such, many commentators were sceptical that the HPLC-UV method (high performance liquid chromatography – ultra violet detection) for example, developed by ANSM (French National Agency for Medicines and Health Product Safety) with a claimed LOQ of 0.3 ppm could reproducibly analyse samples at these very low levels.[97]

However, three methods from the OMCL network did use hyphenated GC (gas chromatography) or HPLC (high performance liquid chromatography) methodology: an HS-GC–MS method (head space-gas chromatography-mass spectroscopic detection) from PALG (Public Analysts in Galway), with an NDMA LOQ of 0.04ppm[98]; an APCI-UHPLC-MS/MS method (atmospheric pressure chemical ionization-ultra-high performance liquid chromatography - mass spectroscopic detection) from CVUA (Chemischen und Veterinäruntersuchungsamt)[99] with an NDMA LOQ in API of 0.2 ppm and in drug product of 0.1 ppm, and an NDEA LOQ in API of 0.08 ppm and in drug product of 0.04 ppm; finally, the HPLC-MS/MS method (high performance liquid chromatography – mass spectroscopic/mass spectroscopic) from LGL (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit)[100] with an NMBA LOQ of 28.6ppb. Although the network subsequently identified these three hyphenated techniques as their "methods of choice" in the development of the European Pharmacopoeia (Ph.Eur.) general chapter 2.4.36 (see below), it is still unclear from the OMCL Network webpage, which methods are considered most appropriate.[101]

The Ph.Eur. in collaboration with the OMCL network is developing a new general chapter on the analysis of N-nitrosamine impurities in active substances (2.4.36).[102] The chapter focuses on the analysis of N-nitrosamines in ARBs (sartans) containing a tetrazole group, for which there are currently five Ph.Eur. monographs (valsartan, losartan potassium, candesartan cilexetil, irbesartan and olmesartan medoxomil). Manufacturers of these five ARBs have to implement a control strategy for N-nitrosamines, and from April 2021 the batches of API they produce must not contain quantifiable levels, corresponding to < 0.03 ppm, i.e. 30 ppb, of the two main N-nitrosamine impurities, i.e. NDMA and NDEA.

General chapter (2.4.36) proposes three procedures using either GC–MS, GC–MS/MS and HPLC-MS/MS covering seven N-nitrosamine impurities: N-nitroso-dimethylamine (NDMA), N-nitroso-diethylamine (NDEA), N-nitroso-dibutylamine (NDBA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA); N-nitroso-diisopropylamine (NDiPA), N-nitroso-ethylisopropylamine (NEiPA) and N-nitroso-dipropylamine (NDPA). The three listed procedures have been validated as limit tests with a target concentration of 30 ppb, for the designated APIs. Ph.Eur. reflected that: "It was considered important to include a varied set of procedures using different instruments, thus covering the needs of many quality control laboratories in Europe and beyond".[102]

As a result of a "Lessons Learnt" exercise, EMA have indicated that in the future, EDQM (European Directorate of Quality of Medicines) will coordinate testing priorities, rather than the individual OMCLs. In addition, all individual OMCLs will have adequate resources to test for trace levels of MIs, including modern, hyphenated chromatographic methodologies, i.e. GC–MS and HPLC-MS.[31]

US Food and Drug administration & USP

In contrast, FDA focused their efforts far more intensely. The original HS-GC–MS method developed for NDMA in APIs had an LOQ of 0.3 ppm.[103] A more sensitive HS-GC–MS for both NDMA and NDEA was subsequently required and had LOQ limits of 0.1 and 0.05 ppm, respectively.[104]

During April 2019, FDA issued a combined direct injection GC–MS/MS method for NDMA, NDEA, NEIPA, NDIPA, and NDBA to address the fact that other N-nitrosamine impurities had being found in valsartan drug substance and drug products.[105] The method showed excellent sensitivity for all N-nitrosamines in the API (LOQ 0.05 ppm) and drug product (LOQ 0.05 ppm). This was supplemented with a headspace GC–MS/MS method for NDMA, NDEA, NEIPA, NDIPA.[106]

FDA also issued an HPLC-HR-MS method (high performance liquid chromatography-high resolution-mass spectroscopic detection) for six N-nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, NDBA and NDBA) in losartan drug substance and drug product at sub-ppm levels (LOQ 0.05 ppm, range: 0.05–5 ppm).[107] This was the first method capable of directly detecting and quantifying NMBA. The FDA indicated that "the method may also be capable of testing for these six impurities in other ARB drug substances and drug products pending verification and/or validation".

FDA also developed and validated a RapidFire-MS/MS method for screening of nitrosamine impurities NDMA, NDEA, NEIPA, NDIPA, NDBA and NMBA in losartan potassium API batches.[108] The method was determined to be accurate, precise, specific and linear over the corresponding analytical ranges, with LOQs of 25 ppm (NDMA), 50 ppm (NDEA), 0.1 ppm (NEIPA), 0.25 ppm (NDIPA), 0.1 ppm (NDBA) and 0.1 ppm (NMBA), respectively. FDA commented that: "This method would not be sufficient for batch release purposes for verifying that NDMA or NDEA is not present in drugs intended for human use".

The findings from the Valisure Citizens Petition on ranitidine,[69] which cited very high levels of NDMA in ranitidine tablets, i.e. 3 mg/tablet, prompted another FDA method re-assessment as it became clear that these elevated levels were a result of ranitidine degradation within the GC injection port leading to artifactually high levels of the analyte. The FDA subsequently developed a HPLC—HR-MS method for NDMA in ranitidine with an LOQ of 0.033 ppm.[70]

A second Valisure Citizens Petition[74] on high levels of NDMA in metformin products, i.e. API, IR (instant release) and ER prompted FDA to caution users on potential method-interference issues. FDA rapidly sought to repeat the analysis of the affected batches. They found that re-testing the samples using two orthogonal methods demonstrated that FDA's results were lower than those reported by Valisure. This was attributed to interference from DMF in the method and that unless a high accuracy MS was used during data acquisition and processing then over-estimation of NDMA was likely (the molecular weight difference between DMF and NDMA is only 0.0016 amu or 21 ppm). FDA subsequently reported that the application of very sensitive methods for routine testing of N-nitrosamines in novel matrices will not be straightforward.[75]

The United States Pharmacopeia (USP) have published a draft general chapter <1469> Nitrosamine Impurities in Pharmacopeial Forum.[109] They report two procedures that can be employed. Procedure one employs HPLC—HR-MS methodology to quantify six nitrosamines, i.e. NDMA, NDEA, NDIPA, NEIPA, NMBA and NDBA, with a sensitivity of 1 ng/mL (i.e. 1ppb). Procedure two employs HS-GC–MS/S methodology for the quantification of four nitrosamines, i.e. NDMA, NDEA, NDIPA, NEIPA and has a sensitivity of 4 ng/mL i.e. 4ppb.

Other contributors

There is a Swissmedic limit test for the determination of N-nitrosamines (NDMA, NDEA, EIPNA, DIPNA, DPNA and DBNA) by GC–MS/MS, which is validated for the five impacted ARBs, i.e. valsartan losartan, irbesartan, olmesartan and candesartan. The method highlights that for other APIs or finished products, that in-situ validation, with a focus on extraction, specificity and quantification will be required.[110] Health Canada developed a method to detect and quantify the nitrosamine impurities NDMA and NDEA in ARBs. The procedure is performed by GC–MS-MS using direct injection. The method cautions that if interference is observed further validation may be required.[111]

The TFDA issued a series of methods over a 2-year period.[112-114] In August 2018 they reported an HPLC-MS/MS method for NDMA and NDEA. This was initially developed for valsartan, before being extended to the other affected sartans, ranitidine and metformin APIs and drug products.[112] During 2019 they released an HPLC-MS/MS method for N-nitroso-N-methyl-4-aminobutyric acid (NMBA) in affected sartan drug substance and their drug products.[113] In late 2019 they issued an HPLC-MS/MS method for twelve N-nitrosamines, i.e. NDMA, NDEA, NDiPA, NDPA, NEIPA, NMBA, NMEA, N-Nitrosodiethanolamine (NDELA), N-Nitrosomorpholine (NMOR), N-Nitrosopiperidine (NPIP), N-Nitrosopyrrolidine (NPYR), N-Nitrosodiisopropanolamine (NDiPLA), in all medicines.[114]

In addition, many instrument manufacturers, academic institutions and companies have published their own methodologies. However, despite the plethora of available methodologies it is still unclear to many industrial users which are the best method(s), particularly against a background of ever lower, "technically"-based limits, i.e. 30 ppb in EU. There appears to be no published account of technology transfer of these highly sensitive methods between different laboratories to demonstrate that they are fit for purpose in other laboratories. Equally, the huge demand for ppb-based methods is threatening to overwhelm world-wide capacity; indeed many CMOs (contract manufacturing organizations) are diverting capacity from bio-analysis to satisfy demand. In addition, there is no clear picture as to whether these ppb-based methods are sufficiently rugged and robust to satisfy ongoing demands.

We already know from the Valisure experiences[69,74] that a method may be inappropriate for the intended matrix, i.e. direct injection GC–MS method for NDMA in ranitidine (originally developed for sartans) or that the method will over-estimate these analytes due to interference or lack of sufficient mass accuracy within the MS detector, i.e. HPLC—HR-MS method[74] for NDMA in metformin products. In the latter case, a second orthogonal method was required to confirm the correct result.[75] This requirement for a second, highly sensitive orthogonal method, will again negatively impact on capacity considerations.

Lastly, it has become apparent that implementing extremely sensitive methodologies, i.e. GC–MS(n) or HPLC-MS(n) to monitor levels of N-nitrosamine impurities in medicinal products at these very low limits is fraught with uncertainty. The major concern is that there are a limited number of global laboratories and support staff who can routinely operate this type of methodology and produce reliable data. As with all trace analysis applications, contamination is a very real issue. The worry is that we will see many more issues of methodology-derived specification failures, i.e. false positives arising from co-eluting contaminant peaks, e.g. DMF, where analytical issues result in acceptable product being recalled from the market. In addition to the financial impact, these product recalls also affect the global supply chain affecting patient access to critical medicines; further undermining the public's confidence in the health system.

Control strategies

In the short term most agencies have set AI-based limits using ICHM7(R1).[4] In the EU, companies were then requested to "make necessary changes to their API-manufacturing processes to minimize nitrosamine contamination", i.e. avoidance, and they were given a 2-year transition period to accomplish this; thereafter, they are required to meet much stricter limits, i.e. 0.03 ppm (30 ppb), which is based on ALARP principles, set on the basis of method sensitivity of those methods validated by OMCL laboratories.[31] So the situation has

Table 3
Commentary on EMA's recommendations on ICH Q7 from Lessons Learnt[31] after action review.

EMA Observations and Recommendations	Commentary on Proposals
EMA would like to amend ICH Q7[116] to restrict the use of "reagents or recovery processes" to the same designated processes or preferably to the identical step from which they were recovered, as this could introduce CoC impurities, particularly for those activities that are subcontracted to third parties. EMA propose to mandate the validation of any recycling processes, particularly those occurring in the final stages of API synthesis.	Restricting the re-use of recovered solvents to the originating step of the designated synthetic pathway and negating their use in the final stages of the synthetic route only makes sense if the risk assessment has identified a potential hazard from N-nitrosamine contamination. As such, it would be inappropriate if there are no identified hazards. This proposal significantly downplays the importance of solvent re-cycling from an environmental perspective. However, using third parties for solvent recovery looks to be a fundamentally bad practice as the MAH has no control of that processing stage and so validation of any recycling stage is considered scientifically justified.
EMA would like to review its own "GMP guideline, Part 1, chapter 7" on outsourcing[118] to better describe how MAHs can take full and complete responsibility for the overall quality of their products, especially where the input drug substance is approved via a certificate of suitability with respect to a Ph.Eur. monograph (CEP) or an active substance master file (ASMF).	Insisting that MAHs take full and complete responsibility for the overall quality of their products makes good scientific sense, but historically this has been stymied by API suppliers. These third parties are reluctant to divulge full details of their processes and any resultant signal impurities because of the justifiable fears that their intellectual property may be compromised. To make this proposal work API suppliers will need to be brought on board.

evolved from control strategies based on safety-based limits, i.e. AIs, to ALARP-based and finally to concentration-based limits established using the technical feasibility of the methodology rather than a virtually safe dose.

The USP in its new general chapter ⟨1469⟩ Nitrosamine impurities has a section on control strategies.[115] The ultimate goal of the control strategy is "ensuring that levels of nitrosamines, if their presence could not be totally avoided, are at or below the provisional acceptable intake (AI)". The section provides guidance on how to achieve the goal using risk assessment, "the components of DP should be assessed for the potential to form nitrosamines or be contaminated with nitrosamines." The section includes a high-level process flow to facilitate the generation of a nitrosamine impurity control strategy.

What can be done?

We are currently awaiting after-action reviews (AARs) from many of the major regulatory authorities, including FDA, PMDA, Health Canada, Swissmedic, etc. However, EMA have issued their "Lessons Learnt" document.[31] The recommendations are fairly wide-ranging and cover many different aspects of manufacturing, packaging and distribution as well as proposing changes to many of the underpinning ICH quality guidance documents, i.e. ICH Q7[116] and ICH Q9,[117] and multi-disciplinary guidance documents, i.e. ICHM7(R1).[4] A series of summaries are provided in Table 3-7.

Conclusions

The identification of NDMA contamination in valsartan from a Chinese supplier in mid-2018 was the beginning of an extremely challenging 24-month period for the pharmaceutical industry. Within a short period of time, the crisis had impacted all sartans medicines containing a tetrazole ring system, global sartan manufacturers (particularly in India and China) and highlighted that the issue went beyond simple NDMA contamination – potentially, nitrosamines originating from all short-chain secondary or tertiary amines can be implicated. Thereafter, other compound classes, e.g. H_2 (histamine-2) blockers, i.e. ranitidine, nizatadine, aminoglyside antibiotics, i.e. rifampine as well as other compounds, e.g. metformin and pioglitazone were also implicated. In addition to intrinsic contamination resulting from the reaction between secondary/tertiary amines and nitrous acid/nitrites, investigations also highlighted GMP concerns arising from contaminated solvents/reagents, degradation of some APIs, e.g. ranitidine, niftazidine and issues with packaging, i.e. lidding foil containing nitrocellulose and excipient concerns, e.g. metformin extended-release drug products. The focus has also recently been extended to biologics/diagnostics, at least in the EU.

So concerned were many global regulators than several mandated risk assessments for all medicinal products in their territories, e.g. Health Canada, Swissmedic, EMA. There were global calls for avoidance: "there is no need to establish AIs for impurities in the first place as the contamination risk is considered to be avoidable by avoiding certain manufacturing processes".[49] Nevertheless, it rapidly became clear that in order to manage the situation in the short-term interim AI limits were necessary. In the longer term, rather than continue to use these interim AIs, which are recognized as virtually safe doses, many regulators have implemented technically based limits, i.e. 30 ppb in EU, that are totally driven by analytical capability and unrelated to classical safety-based limits – a clear case of not tolerating even minimal risk. These strict limits will not apply to oncology products, where currently,

Table 4
Commentary on EMA's recommendations on ICH Q9 from Lessons Learnt[31] after action review.

EMA Observations and Recommendations	Commentary on Proposals
EMA would like to enhance the transparency of ICH Q9[117] to further improve the risk assessment processes. In particular, what constitutes a risk assessment and how risk assessments should be undertaken? This is based on EMA findings for a significant number of MAA submissions, of large numbers of gaps and uncertainties. EMA suggest that this is probably best accomplished using a Q&A document and supporting training resources.	Implementing enhanced risk assessment procedures via a Q&A approach makes good scientific sense, but in the initial valsartan case the risk assessment focused on the significant hazard arising from hydrazoic acid, which poses a dangerous explosion risk when shocked or heated, particularly at large scale. Hydrazoic acid is a category 2 sensitizer, which shows specific target organ toxicity (category 1) for central nervous and cardiovascular systems[119]; as such, it poses a significant worker-safety hazard. Consequently, the less obvious risk posed by the residual $NaNO_2$ was missed, reflecting a common issue with risk assessments, that "risk is in the eye of the beholder". FDA indicated in early 2019, that although they had issued a recent guidance on mutagenic impurities risk assessments in March 2018, that "neither regulators nor industry fully understood how NDMA and NDEA could form during this particular manufacturing process".[47]

Table 5
Commentary on EMA's recommendations on ICH M7(R1) from Lessons Learnt[31] after action review.

EMA Observations and Recommendations	Commentary on Proposals
There are a number of exclusions in ICH M7(R1) guidance[4] and EMA would like to retroactively apply the guidance to all marketed products, as well as taking precautionary measures to lessen the risk of N-nitrosamine formation during the "manufacture and storage" of all drug substances and drug products.	This proposal seems to be non-proportionate when industry has been asked to complete a 3-stage risk assessment process for all marketed medicinal products in many major markets, i.e. EU, Canada, Switzerland, etc. It would be more sensible to retrospectively apply the guidance to those products that are highlighted as posing a high risk.
EMA would like to provide increased transparency for the methodologies that should be used to determine "AI levels for CoC compounds" - particularly N-nitrosamines.	The existing ICH M7(R1)[4] methodology (i.e. process for defining the proposed AI limit) seems to be robust and does generate reproducible AI limits. Are the EMA uncomfortable with the existing AIs as "virtually safe doses" and are looking to implement a lower generic limit, i.e. 30 ppb for all N-nitrosamines in all medicinal products? If EMA does not accept the concept of AIs as suitable limits for N-nitrosamines, there seems to be little utility in clarifying the methodology.
EMA question whether API control options 2, 3, and 4 are appropriate for CoC compounds. EMA have indicated that "the focus on process understanding, impurity fate mapping and impurity purging is strongly endorsed; however, the lack of testing where there is a risk of such toxic impurities being present within an API is not appropriate".	It seems strange that EMA strongly endorses process understanding, impurity fate mapping and impurity purging, but supports a testing -based approach for CoC impurities, i.e. a generic limit of 30 ppb. The physiochemical parameters underpinning purging, impurity fate mapping and indeed process understanding are not affected by the toxicity of the potential impurity. Recent work from Burns et al.[88] on Candesartan, a sartan where the tetrazole ring is introduced earlier into the synthesis (cf. valsartan, where it is introduced in the penultimate stage) shows compelling evidence that the purge is effective in removing N-nitrosamine impurities and this is supported by extensive analytical data to validate the absence of these CoC impurities in the API.
EMA would like to see additional clarification to section 5 of ICH M7(R1),[4] to address potential side reactions for example, "between reagents and solvents (or impurities in solvents) should also be evaluated when considering potential mutagenic impurities".	This proposal makes sense but it is still constrained by our limited knowledge of side reactions from a ppm (or ppb) perspective.
EMA would like to assess whether the list of CoC compounds needs to include additional compounds based on food-safety regulations	Delaney[39] reviewed the typical classes of chemicals that underpin the TTC and compared them with reagents and reactions that are normally encountered in synthetic chemistry and concluded that the existing CoC chemicals are not commonly used. Therefore, it is considered unlikely that food-based CoCs (such as mycotoxins) would necessarily be encountered in synthesis.
EMA would like to publish a Q&A document with detailed information on potential sources of N-nitrosamine impurities and of other CoC impurities (e.g. azoxy compounds) and the process conditions facilitating their formation. EDQM should provide additional recommendations (along with appropriate control strategies) for all drug substances to avoid deliberate or inadvertent introduction of all CoC impurities in APIs used in drug products. This could form part of the revision for the monograph on Substances for Pharmaceutical Use.	This is a proportionate response to the N-nitrosamine contamination issues.

due to the markedly different risk/benefit considerations for cancer patients, mutagenic impurities can be controlled to higher limits than those articulated in ICHM7(R1).[4] Regulatory agencies are silent as to whether other diseases with reduced life expectancy, e.g. rare diseases will be similarly allowed to implement higher limits. Interestingly, FDA is allowing levels of N-nitrosamines in aminoglyside antibiotics, i.e. rifampine above the designated AI-based limits "to maintain patient access to these life-saving medications".

In addition, it has become apparent that implementing extremely sensitive methodology, i.e. GC–$MS_{(n)}$ or HPLC-$MS_{(n)}$ to monitor medicinal products at these very low limits is fraught with uncertainty. Valisure, a US company has issued two Citizens Petitions during this crisis: for rantidine[69] and metformin.[74] In both cases Valisure reported over-inflated levels of NDMA in these products, unnecessarily heightening public unease. The major concern is that there are a limited number of global laboratories and support staff who can routinely operate this type of methodology and produce reliable data. The worry is that we will see many more issues of methodology-derived specification failures, where analytical issues result in acceptable product being recalled from the market, further undermining the public's confidence in the health system.

In Europe, the CHMP in their updated questions and answers summary of the article 5(3) review have also indicated, with little convincing evidence, that the use of less than lifetime (LTL) limits for N-nitrosamines, a mainstay of ICH M7(R1),[4] particularly in early drug development, is not permitted.[30] Thus, ppb methodology will need to be employed for sub-optimal processes for early-phase compounds with a high likelihood of attrition. USP has indicated that, "Nitrosamines are common chemicals in water and foods including cured and grilled meats, dairy products and vegetables. Everyone is exposed to some level of nitrosamines".[115] ICH M7(R1)[4] also indicated that, "Higher acceptable intakes may be justified when human exposure to the impurity will be much greater from other sources e.g., food, or endogenous metabolism (e.g., formaldehyde). Against this background it is surprising that USP (and other regulatory bodies) should indicate that, "However, their presence in medicines, even at trace level is considered unacceptable because nitrosamine impurities are probable human carcinogens".[115]

At the same time, throughout this crisis regulators have sought to ease patient safety concerns by restating that N-nitrosamine exposure is widely encountered via food and water and that it was much more important that patients continued to take their medicines. Recently, FDA[43] reiterated that patients taking aminoglyside antibiotics contaminated with N-nitrosamines should continue taking their medicines based on a risk/benefit assessment. Longer term cancer risk assessments based on taking N-nitrosamine contaminated valsartan for up to 4.6 years showed an excess cancer risk of 1/3390. In a BMJ editorial[53] there was a call for longer-term assessment of exposed patients. Nevertheless, CHMP did not support these proposals. They argued that "theoretical risk of cancer was very low and was itself based on a worst-case scenario".[31] The lay observer has a right to be confused. Either, the theoretical risk of excess risk of cancer is low, in which case AI-based limits, which constitute virtually safe doses are appropriate or the risk to patients is significant and product recalls, avoidance and limits significantly below the AI are applicable. One thing is certain, it cannot be both scenarios.

Risk-based assessments and ppb chemistry knowledge are intrinsically contradictory in nature and as FDA indicated, "neither regulators

Table 6
Commentary on EMA's recommendations on EU's Guideline on Chemistry of Active Substances from Lessons Learnt[31] after action review.

EMA Observations and Recommendations	Commentary on Proposals
EMA recommends that in section S.2.6.of the Common Technical Document (CTD) that industry develops processes that "do not produce CoC impurities", i.e. avoidance or that they minimize the levels by introducing appropriate risk-reduction procedures, i.e. equivalent to ALARP.	The approach initially seems feasible, with the caveat that it may introduce other risks that may be more hazardous than the initial concerns. For example, the majority of tetrazole syntheses in sartan dossiers utilise mutagenic azide reagents, e.g. sodium azide (NaN_3), tributyltin chloride (Bu_3SnCl) /sodium azide or zinc bromide ($ZnBr_2$)/sodium azide, tributyltin azide (Bu_3SnN_3), or triethylammonium chloride (TEA HCl)/sodium azide. A typical drawback of using azide reagents is the potential formation of hazardous hydrazoic acid.[120] In the majority of sartan- manufacturing processes, equimolar quantities (as a minimum) of sodium nitrite are employed to ensure complete removal of the hazardous azide reagents.
EMA indicates that companies should justify the designated manufacturing process by discussing the formation (real or potential) of mutagenic impurities (real or potential), particularly CoC impurities. They further state that if the use of nitrosating agents cannot be avoided, then the potential combination with secondary amines under those conditions known to favour N-nitrosamine formation should be avoided unless appropriately justified. Further, that such justification could include Ames testing or "nitrosatability testing, i.e. WHO NAP test"[121] of any potential N-nitrosamine impurities.	This request could prompt regulators to adopt an "avoidance mind-set" for all mutagenic impurities, not just CoC impurities. As Elder & Teasdale[6] clearly demonstrated, avoidance of all MIs is not tenable, whereas avoidance of CoC impurities may be.
EMA indicated that the guideline should mandate that all the processing materials (e.g. starting materials, reagents, catalysts, processing aids, solvents, gases and materials used during work-up, i.e. quenching agents) utilized should be divulged in sections S.2.2 and S.2.3 of the CTD. Further, that these materials should be unambiguously ascribed to the appropriate step or sub-step, together with the intended function of these materials and that in section S.2.2, the molar equivalents of all reagents, catalysts and quenching agents should be clearly stated referencing the appropriate starting material in the designated manufacturing step. Importantly, the use of such materials in molar excess needs to be adequately justified and supported unless this is established as normal practice, e.g., use of excess NaOH in ester hydrolysis reactions.	This is a proportionate response to the N-nitrosamine contamination issues.
EMA indicated that guidance should be provided to applicants on the potential contamination of raw materials (particularly solvents and reagents) with nitrosating agents, e.g., $NaNO_2$, which can be inadequately purged in subsequent downstream stages of chemistry. Suitable acceptance criteria should be defined and appropriately justified by spike/fate and purge studies. EMA recommended that the impurities section needed to be updated to highlight the "new methodical approach" introduced in ICH M7(R1)[4] for mutagenic impurities consisting of hazard evaluations of all organic impurities ((Q)SAR and literature searches for both carcinogenicity and bacterial mutagenicity alerts) to classify impurities as non-mutagenic (category 4 or 5) or mutagenic (category 1 or 2) and application of the corresponding appropriate acceptance criteria. EMA commented that presently, applicants typically utilise an invalid, "outdated methodology" to identify those alerting structures.	Applying ICHM7(R1)[4] hazard assessments to all organic impurities within the process does seem excessive, as there is a clear structural correlation between functional alerting moieties and mutagenicity, which underpins (Q)SAR assessments. A more pragmatic approach, which combines the use of literature data for impurities such as dimethylamine or diethylamine and (Q)SAR assessment, with follow-up expert review, for impurities lacking experimental mutagenicity data, would appear to be more tenable.
Finally, EMA seeks to provide applicants with guidance on the quality and supporting specifications of starting materials of drug substances and registered intermediates that may be contaminated with N-nitrosamine impurities. They seek to recommend to applicants additional guidance for relevant controls for CoC impurities under section S.3.2. of the CTD.	This is a proportionate response to the N-nitrosamine contamination issues.

nor industry fully understood how NDMA and NDEA could form during this particular manufacturing process".[47] In addition, risk is often in the eye of the beholder particularly when a significant process-chemistry risk masks the potential for another underlying risk. The valsartan contamination problem arose because $NaNO_2$ was used as an azide-quenching agent to address the significantly more hazardous (in the minds of the risk assessors) formation of hydrazoic acid, which poses a dangerous explosive risk when shocked or heated.

Lastly, although some of the proposed fixes for the problems seem logical and even-handed, many more reflect regulators' unease in risk-based resolutions; they want analytical data to re-assure themselves. This crisis highlights a fundamental unwillingness to tolerate risk from pharmaceutical products, even when we are exposed to the same risk on a daily basis through diet and drinking water.

Abbreviations

PDE (permissible daily exposure); AI (acceptable intake); less than lifetime (LTL) limits; TTC (threshold of toxicological concern); VSD (virtually safe dose); ALARP (as low as reasonably practicable); Active Pharmaceutical Ingredients (APIs); International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH); Cohort of Concern (CoC); Quantitative Structure Activity Relationships ((Q)SAR); Mutagenic Impurities (MI); Potentially Mutagenic Impurities (PMIs); Poly Aromatic Hydrocarbons (PAHs); DeoxyRibonucleic acid (DNA); Cytochrome(CYP); Benchmark Dose Modelling (BMDL10); Point of Departure (PoD); Dose that results in a 50% increased tumour incidence over background median toxic dose (TD_{50}); Carcinogenic Potency Database (CPDB); NMDA (N-nitrosodimethylamine); N-Nitrosodiethylamine (NDEA); N-Nitroso-N-methylamino butyric acid (NMBA); N-Nitrosodipropylamine (DPNA); N-Nitrosodiisopropylamine (DIPNA); N-Nitrosoethylisopropylamine (EIPNA); N-nitroso-N-methylaniline (NMPA); 1-methyl-4-nitrosopiperazine (MNP); 1-cyclopentyl-4-nitrosopiperazine (CPNP); N-Nitrosodiethanolamine (NDELA); N-Nitrosomorpholine (NMOR); N-Nitrosopiperidine (NPIP); N-Nitrosopyrrolidine (NPYR); N-Nitrosodiisopropanolamine (NDIPLA); Latent Variable Models (LVM); Margin of Exposure (MOE); SCCS's (Scientific Committee on Consumer Safety); Committee for Medicinal Products for Human use Safety Working Party (CHMP's SWP); US Food and Drug Administration (FDA);

Table 7
Commentary on EMA's recommendations on EMA GMP guidelines from Lessons Learnt[31] after action review.

EMA Observations and Recommendations	Commentary on Proposals
EMA wants to remind MAHs of their overall responsibilities for the quality of the medicinal product. There should be enhanced quality agreements in place between MAHs and drug-product manufacturers, rather than just purchasing agreements and APIs should not be viewed as commodity chemicals. They seek to improve quality audits of API manufacturers by MAHs and to enhance the role of the qualified person to enhance the quality-management system of MAHs. Similarly, EMA seeks to improve the CEP system by increasing transparency, to better understand and comply with the needs of MAHs and regulators. This should include improved awareness of the MAH's responsibilities in those cases where the API is covered by a CEP and importantly improve the awareness of CEP holders' responsibilities to the MAHs, particularly regarding accessibility of API information within the regulatory dossier. The information provided to the MAHs should be equivalent to that required by an ASMF, with respect to manufacturing process and impurities. The ASMF process also needs updating to improve transparency to the MAH on process-related impurities, including awareness on the materials used in synthesis.	As stated earlier, this recommendation relies on the API supplier being willing to divulge proprietary information to a third party and circumventing the existing CEP and ASMF processes (and drug master files in the US), which provide a strategy for divulging this information directly to the regulatory authorities.
EMA recommends that, in addition, it should consider mandating that MAHs "generate and submit their own information on impurities rather than just relying on information provided by their API suppliers".	Many small companies do not have the resources or the technical expertise to "generate and submit their own information". They are totally reliant on the API supplier to provide such data.
EMA indicates that the conditions/documentation for adding or changing API manufacturers and manufacturing processes within ASMFs and CEPs, need to be clarified to avoid misclassifications and to assess the impact of such changes on drug product quality. The MAH should have appropriate knowledge of the quality of any new source of API covered by a CEP.	It is debatable whether many of the virtual or smaller MAHs would have the appropriate understanding to assess the impact of these API changes on drug product quality. Indeed, it is doubtful whether many of the smaller drug-product CMOs would have that knowledge either.

European Medicines Agency (EMA); Central Drugs Standard Control Organization (CDSCO); National Medical Product Administration (NMPA); Japan's Ministry of Health, Labour and Welfare/Pharmaceuticals and Medical Devices Agency (MHLW); Australia's Therapeutic Goods Administration (TGA); Singapore's Health Science Authority (HSA); British Medical Journal (BMJ); Taiwanese Food and Drug Administration (TFDA); Dimethylformamide (DMF); Angiotensin II Receptor Blocker (ARB); Certificate of Suitability to the monographs of the European Pharmacopoeia (CEP);European Directorate of Quality of Medicines (EDQM); Marketing Authorisation Holders (MAHs); NCAs (National Competent Authorities); Dimethylamine (DMA); Diisopropylethylamine (DIPEA); trimethylamine (TEA); diethylamine (DEA);: 4-methylaminobutryic acid (MBA); N,N-dimethylaniline (N,N-DMA); dibutylamine (DBA); tributylamine (TBA); Good Manufacturing Practice (GMP); Tetrabutylammonium bromide (TBAB); World Health Authority (WHO); European Pharmacopoeia (Ph.Eur.); United States Pharmacopeia (USP); United States (US); Official Medicines Control Laboratories (OMCL); French National Agency for Medicines and Health Product Safety (ANSM); Gas Chromatography (GC); High Performance Liquid Chromatography (HPLC); Head Space- Gas Chromatography-Mass Spectroscopy (HS-GC–MS); Public Analysts in Galway (PALG); Atmospheric Pressure Chemical Ionization- Ultra-High Performance Liquid Chromatography - Mass Spectroscopy (APCI-UHPLC-MS/MS); Chemischen und Veterinäruntersuchungsamt (CVUA); High Performance Liquid Chromatography - Mass Spectroscopy/Mass Spectroscopy (HPLC-MS/MS); Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL); Limit of quantification (LOQ); High Performance Liquid Chromatography-High Resolution-Mass Spectroscopy (HPLC-HR-MS); Parts –per-Million (ppm); IParts –per-Billion (ppb); Instant release (IR); Extended Release (ER); Contract Manufacturing Organisations (CMOs); After-Action Reviews (AARs); Marketing Application Authorisation (MAA); Questions & Answers (Q&As); Active Substance Master File (ASMF); Common Technical Document (CTD); European Union (EU); Zhejianjg Huahai Pharma (ZHP); Zhejiang Tianyu Pharma (ZTP); Zhuhai Rundu Pharma (ZRP).

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Addendum

During February 2021, two major new guidance documents were issued. In early, February, FDA issued updated guidance on "Control of Nitrosamines in Human Drugs"[122] and later the same month (February 22, 2021), EMA released a document (EMA/425645/2020)[123] setting out "measures to limit the presence of N-nitrosamines in human medicines as far as possible and to ensure levels of these impurities do not exceed set limits." These documents were released during the review process of our commentary on this subject: Tolerability of risk: a commentary on the nitrosamine contamination issue (published on line, March 8, 2021), as such we were not able to review these specific documents. This addendum describes how the process in both the US and Europe have evolved further from our initial commentary.

FDA took the very unusual step of implementing the revised guidance without seeking prior public comment, "because of the importance of providing timely information to manufacturers regarding risk assessments, testing, and other appropriate actions they should take to reduce and mitigate nitrosamine impurities in active pharmaceutical ingredients and drug products." The FDA have now adopted the three-stage risk assessment process in common with many other regulatory agencies. They have summarized likely causes of N-nitrosamine contamination to facilitate the risk assessment process. They have implemented a total limit of N-nitrosamines (in those cases where more than one N-nitrosamine is present) of 30 ppb, for drug products with a maximum daily dose (MDD) of < 880mg/day, which equates to a total limit not exceeding 26.5 ng/day. If more than one N-nitrosamine is detected the analytical method should be vaidated to ensure the limit of quantitation (LOQ) is < 30 ppb to accurately quantify total nitrosamine levels at "not more than 26.5ng/day". In addition, "if the MDD is 1200 mg, the LOQ should be below 0.02 ppm."

The technical part of the EMA document is focused on procedures for setting limits for N-nitrosamine impurities in human medicinal products.

If only a single N-nitrosamine impurity is present, the AI is employed as a point of reference - determined by linear extrapolation of its TD_{50} (if available) at a risk level of 1 in 100,000. AIs derived by EMA are shown in Table 1. Values for NDEA and NDMA are 26.5 and 96 ng/day respectively.

If more than one N-nitrosamine impurity is detected, two approaches can be used to set limits:

- the total daily intake of all identified N-nitrosamines should not exceed the limit of the most potent N-nitrosamine identified;
- the total risk level of the sum of all detected N-nitrosamines should not exceed a 1 in a 100,000 lifetime risk.

In practice actual limits are determined according to four different scenarios building on the AI of the most potent N-nitrosamine present, and approaches 1 and 2 shown above.

Consider as an illustrative example a valsartan drug product whose maximum daily dose is 320 mg which may contain NDEA as a single impurity or NDMA as an additional impurity.

- NDEA as a single impurity: the concentration of impurity triggering the submission of a variation application is 10% of the AI, 2.65 ng/day, equivalent to 8.3 ppm NDEA.
- NDEA and NDMA both present: the trigger concentration would still be 8.3 ppb but for the sum of the two nitrosamines. Thus, if either nitrosamine were present at only 10% of the total, an analytical method with a limit of quantitation (LoQ) of 0.83 ppb would be needed.

Thus, it is clear that reliable quantification of N-nitrosamine impurities to low-ppb or sub-ppb levels will be required in order to comply with EMA's risk-assessment procedures. These levels are significantly below the previous limit-test target concentration of 30 ppb, which may represent an extremely difficult, if not insurmountable, analytical challenge.

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